CMP-NeuAc and five other cofactors. Five enzymes act under the same conditions without product inhibition, and a separate synthesis of CMP-NeuAc is not necessary. The enzymes might be immobilized and recovered for reuse. The system should be applicable to many other sialyltransferase-catalyzed syntheses of sialosides. Since stereocontrolled sialylation is still a difficult problem in synthetic carbohydrate chemistry,[13] the enzymatic method based on sialyltransferases will obviously become an effective and practical option. With the increasing availability of glycosyltransferases through cloning techniques,[14] enzymatic methods for oligosaccharide synthesis will obviously complement the chemical methods that have already been well established and vigorously practiced by many elegant approaches.[15]

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Supplementary Material Available: Experimental details of cloning, expression, and isolation of CMP-NeuAc synthetase and ^{1}H NMR spectrum of Neuα2,6Galβ1,4GlcNAc (5 pages). Ordering information is given on any current masthead page.

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Steric Course of the Reduction of Ethyl Coenzyme M to Ethane Catalyzed by Methyl Coenzyme M Reductase from *Methanosarcina barkeri*

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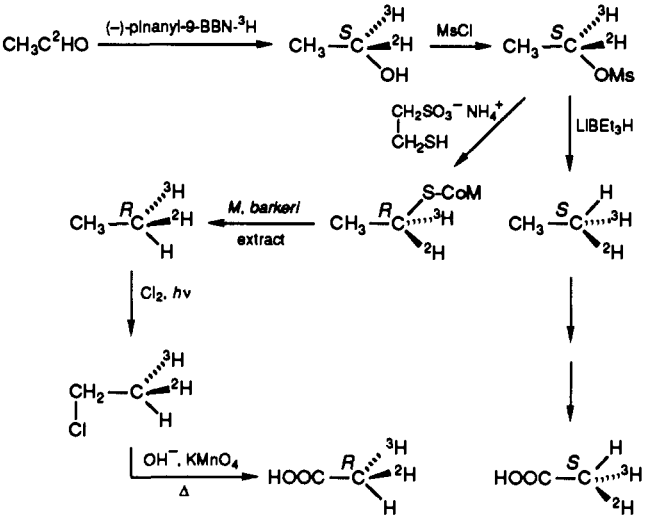
Methanogenic bacteria derive their energy from the reduction of CO_2 with hydrogen gas to methane.[1,2] The terminal step in this sequence, the reduction of methyl coenzyme M to methane, is catalyzed by methyl coenzyme M reductase,[2,3] a highly complex, multicomponent enzyme system[2,4] containing several new cofactors, including the novel nickel-containing tetrapyrrole, cofactor F_{430}[5] (cf. ref 4). Model studies[6] suggest that cleavage of the methyl–sulfur bond of CH_3S-CoM by attack of reduced (Ni^I) F_{430} leads to transfer of the methyl group to the nickel of the cofactor, leaving behind a heterodisulfide with component B of methylreductase (7-mercaptoheptanoyl threonine phosphate), from which coenzyme M is reductively regenerated.[3,7] Methane then arises by protonolysis of the methylated cofactor (CH_3–Ni–F_{430}).[6] The methylreductase complex seems to be located in a membrane-associated particle, the methanoreductosome,[8] which couples reductive methane formation to the generation of a transmembrane proton gradient used by the cell for ATP synthesis.

Scheme I



To test the proposed mechanism for the methylreductase reaction, we decided to determine the steric course of this process. Doing so with methyl-CoM as substrate presents an obvious problem: Four isotopes of hydrogen would be required to generate an isotopically chiral version of methane, but only three hydrogen isotopes are known. Consequently, a different group must be introduced to take the place of the fourth hydrogen isotope. We opted for a methyl group, i.e., we decided to use as substrate ethyl-CoM, which is known to be reduced to ethane at about 20% of the rate of methane formation from methyl-CoM.[9] (*R*)- and (*S*)-[1-$^2H_1$,^{3}H]ethyl coenzyme M were synthesized as shown in Scheme I. Reduction of [1-$^2H_1$]ethanal (98% ^{2}H) with tritiated (+)- and (−)-*B*-(3-pinanyl)-9-borabicyclo[3.3.1]nonane[10] (sp radioact. 5 mCi/mmol, >99% ee) gave (*R*)- and (*S*)-[1-$^2H_1$,^{3}H]-ethanol, respectively.[13] Conversion of each sample to the mesylate was followed by reaction with a solution of 2-thioethanesulfonic acid (coenzyme M) in dilute ammonium hydroxide[9] to produce (*S*)- and (*R*)-[1-$^2H_1$,^{3}H]ethyl coenzyme M (sp radioact. 1.4 mCi/mmol and 0.8 mCi/mmol, respectively) in 17.4 and 12.4% of the theoretical overall yield. An aliquot of the intermediate (*S*)-[1-$^2H_1$,^{3}H]ethyl mesylate was reacted with Super-Hydrid, and the resulting ethane was degraded to [2-$^2H_1$,^{3}H]acetic acid as described below. Configurational analysis[14,15] of this acetic acid sample gave an *F* value[16] of 28.1, corresponding to 75% ee *S* isomer,[17] establishing a maximum value for the optical purity of

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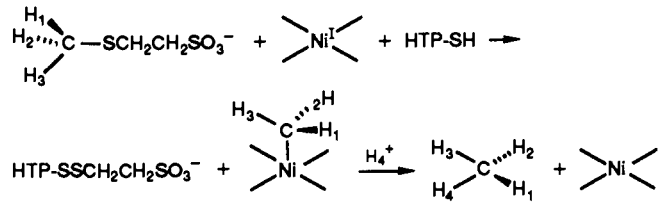
Scheme II[a]



[a] HTP-SH = 7-mercaptoheptanoyl threonine phosphate.

the isotopically chiral methylene group of the ethyl coenzyme M samples.[18]

The samples of (*R*)- and (*S*)-[1-$^2H_1$,^3H]ethyl coenzyme M (1.6 μCi and 2.8 μCi, 2 mM) were each incubated under H_2 gas with an anaerobically prepared cell-free extract[19] of *Methanosarcina barkeri* (1 mL) and 12 mM ATP in crimp-sealed 10-mL vials overnight at 37 °C on a water bath shaker. GC analysis of the head space revealed the formation of 564 nmol of ethane per vial from the *R* samples and 206 nmol from the *S* isomers. The samples were stored frozen prior to workup.

To convert the ethane samples into acetic acid for configurational analysis, the liquid was removed from the thawed reaction vessels with a syringe, and the vessels were rinsed twice with 1 mL of water each. Carrier ethane gas (2.2 mL) and chlorine gas (22 mL) were then introduced into each vial with a gas-tight syringe, and the vials were illuminated for 20 h at room temperature with a UV lamp (254 nm). KOH (0.4 g) dissolved in 5 mL of 5% aqueous $KMnO_4$ solution was added to each vial, and the vials were heated to 120 °C for 24 h. The excess $KMnO_4$ was destroyed with sodium bisulfite. Steam distillation of the alkaline solution then removed impurities, followed by recovery of the acetic acid by steam distillation of the acidified (10% H_2SO_4) solution. The second steam distillate was adjusted to pH 9 with NaOH and evaporated to dryness to give sodium acetate in about 2-3% radiochemical yield.[20] The samples were then subjected to configurational analysis by the method of Cornforth et al.[14] and Arigoni and co-workers,[15] using a procedure employed routinely in this laboratory.[17]

The analyses gave an *F* value of 38.1 for the acetate from (*S*)-[1-$^2H_1$,^3H]ethyl coenzyme M, corresponding to 41% ee *S* configuration; conversely, the acetate from (*R*)-[1-$^2H_1$,^3H]ethyl coenzyme M gave an *F* value of 64.8, corresponding to 51% ee *R* configuration. It follows that the reductive replacement of the sulfur of coenzyme M by a hydrogen in the methylreductase reaction proceeds with net inversion of configuration. This result is consistent with and supports the proposed sequence of events at the alkyl group in the reductive methane formation catalyzed by this enzyme (Scheme II). Displacement of the sulfur from the methyl (ethyl) group by Ni^I presumably proceeds with inversion of configuration, and the subsequent protonolytic cleavage of the alkyl-metal bond, on the basis of existing precedent,[21] would be expected to proceed in a retention mode.

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(18) The low optical purity is probably due to the presence of traces of [^3H]$NaBH_4$ in the [^3H]-9-BBN preparation. When benzaldehyde was reduced with another preparation of (+)-[^3H]pinanyl-9-BBN and the alcohol converted to the (*S*)-mandelate ester, ^3H NMR revealed the presence of (*R*)- and (*S*)-benzyl alcohol in the reduction product in a ratio of 79.4:20.6 (58.8% ee *R*), whereas the reduction of [7-$^2H_1$]benzaldehyde with unlabeled pinanyl-9-BBN is completely stereospecific within the limits of detection.[13]

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Molecular Recognition of Bivalent Sialosides by Influenza Virus

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Infection by influenza virus is initiated by the binding of virus to cell-surface glycoproteins and glycolipids that terminate in sialic acid (SA).[1,2] This interaction is mediated by the trimeric viral protein hemagglutinin (HA).[3] Crystallographic studies of the complex between sialyllactose and hemagglutinin have defined the binding site and confirmed that HA simply recognizes the terminal sialoside of cell-surface oligosaccharides;[3] yet monovalent sialosides bind only weakly, whether to whole virus as measured by the inhibition of erythrocyte agglutination[2] or to bromelain-released HA (BHA) as studied by ^1H NMR.[4] Indeed, there are no reported monovalent α-sialosides with K_d values <2 mM. The binding of virus to cells presumably involves many HA trimers and many sialoside ligands.[5-7] We have investigated two families of bivalent sialosides and find that bis-sialosides of appropriate length bind tightly, not to isolated BHA, but to intact virus. *These ligands evidently bind intermolecularly to adjacent hemagglutinin trimers on the viral surface*, illustrating the energetic consequences of multivalent binding.[5-7]

The binding to BHA of bidentate sialosides having polyethylene glycol linkers (Figure 1A) was shown by NMR titration to be no better than that of the prototypical monovalent ligand, α-methyl-*N*-acetylneuraminic acid (Neu5Acα2Me), which has a K_d of 2.8 mM.[4] Even the longest of these bivalent species, P(5,5) (Figure 1A), which model building suggests is long enough to span two sites on an HA trimer, shows no decrease in K_d. This low affinity has one of two causes. Either the linker is too short (or conformationally too inflexible) to permit both SA residues si-

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